Supporting Information

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SI Materials and Methods

Induction of Transfer EAE. Transfer EAE was induced by transferring 2×10^6 encephalitogenic T cells intravenously into WT sex-matched C57BL/6 mice irradiated with 3.5 Gy. To obtain encephalitogenic T cells, spleen and axillary, inguinal, brachial, and paraaortal lymph nodes were isolated from $\beta_1^{fl/fl}$/2D2$^+$/MxCre$^+$ mice that had received a single intraperitoneal injection of 250 μg of poly(IC) (Amersham Biosciences) in PBS. Single-cell suspensions were obtained, and after ACK lysis of red blood cells (1), cells from 2 mice were cocultured with 4×10^6 DCs loaded for 2 h with 20 μg/mL MOG_{35-55} peptide (Biotrend). After 4 days of coculture, the cell suspensions were split 1:2 into medium containing 5 ng/mL IL-2 (R&D Systems). After another 7 days of culture, dead cells were removed by Nycoprep 1.077 A (Axis-Shield) density gradient centrifugation. For depletion of β_1-positive T cells, the cell suspension was subsequently incubated with a biotinylated β_1-integrin antibody, followed by streptavidin-microbead separation according to the manufacturer's protocol (Miltenyi Biotec). A total of 2×10^6 cells per well were incubated overnight in 24-well plates coated with 1 μg/mL anti-mouse CD3e and CD28 antibodies and in the presence of recombinant murine IL-18 (20 ng/mL; MBL), IL-12 (25 ng/mL), and IL-23 (10 ng/mL; both from R&D Systems). The cells were subsequently washed 3 times with PBS and transferred intravenously into recipient mice irradiated with 3.5 Gy. Clinical disease score and weight of animals with active and transfer EAE were checked daily, and scoring of the mice was as follows: 0, healthy; 1, limp tail; 2, hind leg weakness; 3, one paralyzed hind leg; 4, both hind legs paralyzed; and 5, moribund or dead. Mice scored 1 to 2 were used as recipients for IVM experiments.

Cell Culture. Activated T cells for adhesion assays and IVM experiments were generated in vitro from $\beta_1^{fl/fl}$/OT-II.2$^+$/MxCre$^+$ mice. Mice received a single intraperitoneal injection of 250 μg of poly(IC) in PBS. At 6 to 8 weeks after knockout induction, mice were killed, and single-cell suspensions of splenocytes were obtained. After ACK lysis, splenocytes from 1 mouse were cocultured with 4×10^6 DCs loaded for 2 h with 20 μg/mL $OVA_{323-339}$ peptide (in-house peptide synthesis service). After 4 days of coculture, the cell suspensions were split 1:2 into fresh medium containing 5 ng/mL IL-2 (R&D Systems). After another 2 days of coculture, dead cells were removed by density gradient centrifugation, and β_1-positive T cells were depleted as described above. The efficiency of sorting was monitored by staining with fluorochrome-labeled CD4 and β_1 antibodies and subsequent FACS analysis. On average, 90.4% of the cells were CD4$^+$ and 92.2% of the β_1-depleted cells were β_1-negative.

IVM. IVM images were captured by using a custom-made Mikron IVM500 fluorescence microscope connected to an SIT camera (Dage–MTI). Epiillumination techniques were used to visualize the spinal cord microvasculature after injection of 0.1 mL of 1% TRITC-conjugated dextran (M_r = 155,000; Sigma–Aldrich). Real-time observations were made by using $\times$4, $\times$10, and $\times$20 long-distance working objectives, and microscopic images were recorded by using a DSR-11 digital videocassette recorder (Sony) for later offline analysis, which was performed exactly as described previously (2, 3). $\beta_1^{-/-}$ and control CD4$^+$ T cells, isolated as described above, were fluorescently labeled with 125 nM Calcein-AM (Molecular Probes) before their infusion via the right carotid artery. For direct comparison of the interaction of $\beta_1^{-/-}$ and control T cells within the same spinal cord white matter vascular bed, injection of $\beta_1^{-/-}$ T cells was followed by injection of control T cells (each 4×10^6 cells in 300 μL of 0.9% NaCl injected in aliquots of 100 μL) into the same mouse 1 h later. This experimental setup might produce artefacts because of the extended observation time. Thus, the interaction of $\beta_1^{-/-}$ and control CD4$^+$ T cells was additionally compared in individual mice injected with 1 T cell population only. The same results were obtained using both experimental approaches.

T cells passing through spinal cord microvessels (20–60 μm) and T cells, which visibly initiated contact with the spinal cord microvascular endothelium and thus moved at a slower velocity than the main bloodstream, were counted during an observation period of 1 min in frame-by-frame analysis of the videos using the CapImage software (version 8.3) (2). The fraction of T cells initiating contact with the vascular wall was calculated for each microvessel as the percentage of interacting T cells among the total number of T cells passing through a given postcapillary venule during this 1-min observation window. The rolling fractions and the capture fractions were counted accordingly. Permanently adherent T cells were identified as cells stuck to the vessel wall without moving or detaching from the endothelium within an observation period of $\geq$20 sec and were counted 10, 30, and 60 min after T cell infusion.

1. Bungartz G, *et al.* (2006) Adult murine hematopoiesis can proceed without β1 and β7 integrins. *Blood* 108:1857–1864.
2. Vajkoczy P, Laschinger M, Engelhardt B (2001) *a*4-integrin-VCAM-1 binding mediates G protein-independent capture of encephalitogenic T cell blasts to CNS white matter microvessels. *J Clin Invest* 108:557–565.
3. Stein JV, *et al.* (1999) L-selectin-mediated leukocyte adhesion in vivo: Microvillous distribution determines tethering efficiency, but no rolling velocity. *J Exp Med* 189:37–50.

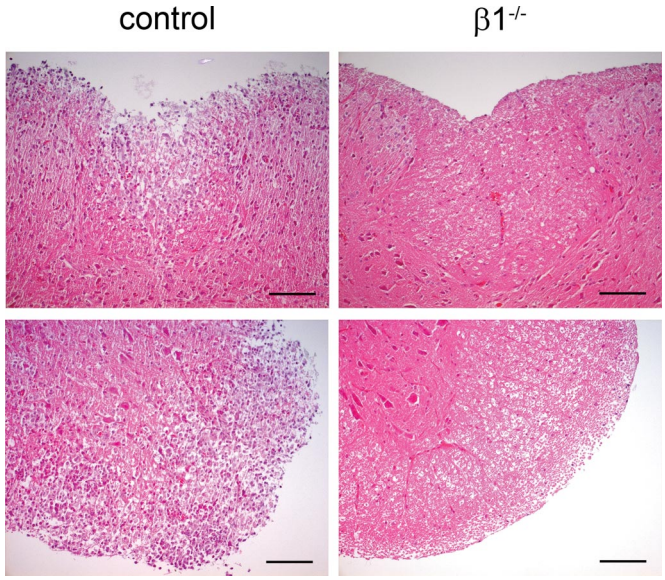


Fig. S1. β_1-Deficient T lymphocytes are not encephalitogenic. Shown are H&E-stained cross-sections of the dorsal part (*Upper*) or the ventral white matter (*Lower*) of the spinal cord of WT mice that received control or $\beta_1^{-/-}$, MOG_{35-55}-specific T cell blasts. (Scale bars, 100 μm.)

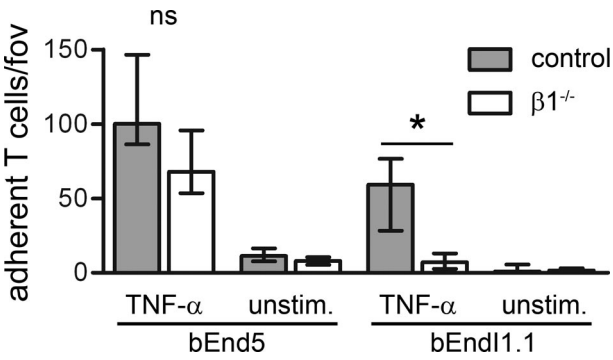


Fig. S2. Static adhesion of T cells to endothelioma cell lines is mainly integrin $\alpha_L\beta_2$-dependent. Adhesion of T cell blasts to the endothelioma cell lines bEnd5 (WT) and bEndI1.1 (ICAM-$1^{-/-}$) was analyzed. Endothelioma cells were stimulated with TNF-α before the assay, which was performed at 4 °C to reduce the contribution of the interaction between LFA-1 and ICAM-1. Graphs show medians and interquartile ranges ($n = 4$).

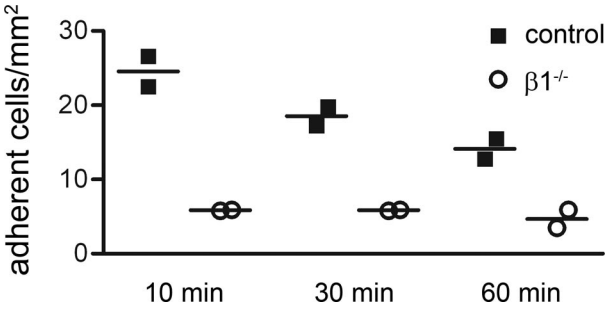
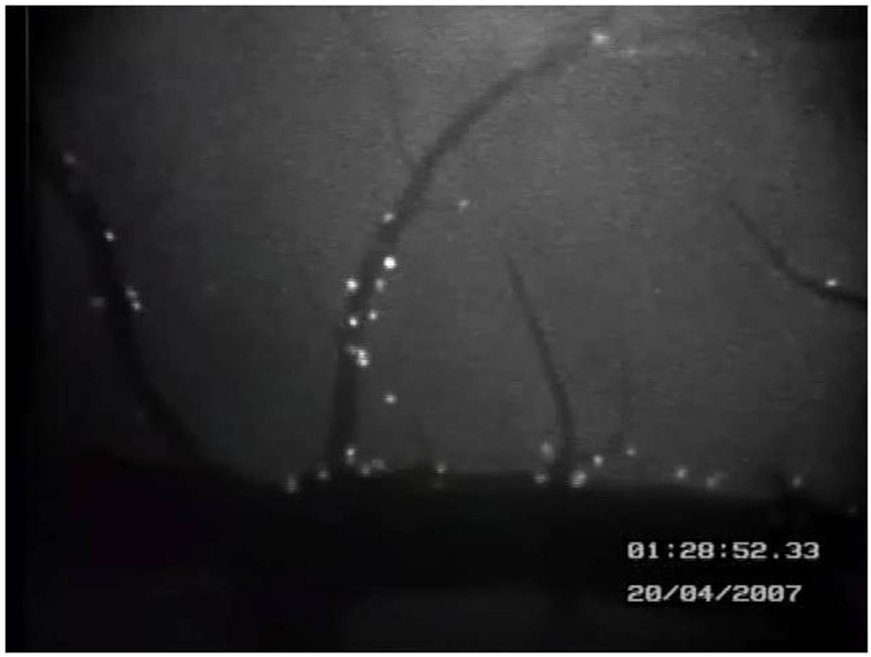
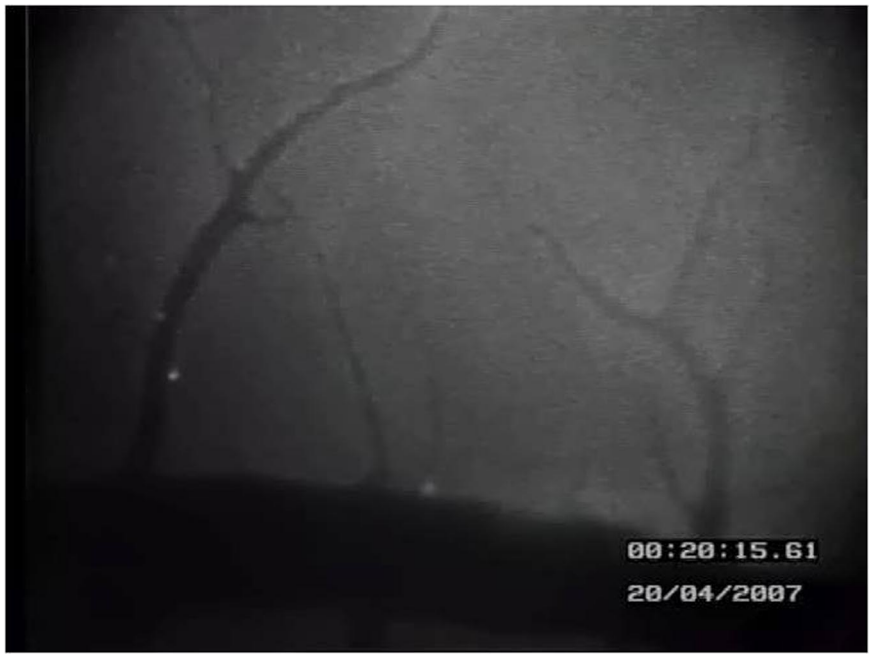


Fig. S3. In vivo firm adhesion of $\beta_1^{-/-}$ T lymphocytes to the spinal cord microvasculature is dramatically reduced. Proliferating $CD4^+$ 2D2 transgenic control and $\beta_1^{-/-}$ T lymphocytes were analyzed for their adhesive behavior in vivo. Firm adhesion of control and $\beta_1^{-/-}$ T cell blasts to the spinal cord microvascular wall was analyzed by intravital microscopy 10 min, 30 min and 1 h after infusion. Each dot represents the mean of one experiment, lines indicate the medians (n = 2).



Movie S1. In vivo adhesion of control T cells to the spinal cord microvasculature. Proliferating $CD4^+$ OT-II.2 transgenic control T lymphocytes were analyzed for their adhesive behavior in vivo. The interaction of T cell blasts with endothelial cells was observed by intravital microscopy of the spinal cord white matter microvasculature in WT mice with ongoing active EAE. Movie shows first the injection of the Calcein-AM-labeled lymphocytes into the microvasculature of anesthetized mice. Adherent T cells 10 min and 1 h after infusion are shown.

Movie S1 (MOV)



Movie S2. In vivo adhesion of $\beta 1^{-/-}$ T cells to the spinal cord microvasculature. Proliferating $CD4^{+}$ OT-II.2 transgenic $\beta_1^{-/-}$ T lymphocytes were analyzed for their adhesive behavior in vivo. The interaction of T cell blasts with endothelial cells was observed by intravital microscopy of the spinal cord white matter microvasculature in WT mice with ongoing active EAE. Movie shows first the injection of the Calcein-AM-labeled lymphocytes into the microvasculature of anesthetized mice. Adherent T cells 10 min and 1 h after infusion are shown. Both movies show the vasculature of the same mouse, into which first $\beta_1^{-/-}$ and then control T cells were injected.

Movie S2 (MOV)

Table S1. Antibodies, specifying label, clone, and distributor

Antigen	Label	Clone	Company
CD28	None	37.51	BD Pharmingen
CD3e	None	145-2C11	BD Pharmingen
CD4	FITC	H129.19	BD Pharmingen
CD4	PE	H129.19	BD Pharmingen
CD4	Biotin	H129.19	BD Pharmingen
CD8	Biotin	53-6.7	BD Pharmingen
Gr-1	PE	RB6-8C5	BD Pharmingen
Hamster IgG	PE	HTK888	BioLegend
Hamster IgM	FITC	G235-1	BD Pharmingen
IFN-γ	PE	XMG1.2	BD Pharmingen
IL-17	PE	TC11-18H10.1	BD Pharmingen
IL-2	PE	JES6-5H4	BD Pharmingen
IL-4	PE	11B11	BD Pharmingen
Integrin α_4	Biotin	9C10	BD Pharmingen
Integrin α_5	Biotin	5H10-27	BD Pharmingen
Integrin α_6	PE	GoH3	BD Pharmingen
Integrin α_E	PE	M290	BD Pharmingen
Integrin α_L	Biotin	M17/4	eBioscience
Integrin α_V	PE	RMV-7	BD Pharmingen
Integrin β_1	Biotin	Ha2/5	BD Pharmingen
Integrin β_1	FITC	Ha2/5	BD Pharmingen
Integrin β_1	PE	HMbeta1—1	BioLegend
Integrin β_2	Biotin	C71/16	BD Pharmingen
Integrin β_7	PE	M293	BD Pharmingen
Ly5.1	Biotin	A20	BD Pharmingen
Mac-1	Biotin	M1/70	BD Pharmingen
Mac-1	Biotin	M1/70	BD Pharmingen
Pan-laminin	None	L9393	Sigma
Rat IgG1	PE	R3–34	BD Pharmingen
Rat IgG2a,κ	PE	R35–95	BD Pharmingen
Rat IgG2a,κ	Biotin	Not available	eBioscience
Rat IgG2b	PE	eB149/10H5	eBioscience
TNF-α	PE	MP6-XT22	BD Pharmingen
Vα 2 TCR	Biotin	B20.1	eBioscience

PE, phycoerythrin.